Remarks

A. General Comments

The instantly claimed invention discloses methods to elicit antigen presentation by cells which normally do not carry out that function and to enhance and improve antigen presentation in cells that do carry out that function such as dendritic cells. It teaches generally how to cause an increase in genes important in antigen presentation. by introducing sequence non-specific double stranded polynucleotides to cells. Among the objects of the invention is to increase the expression of immune response recognition molecules, and to exploit the expression for the treatment of specific diseases.

The Examiner stated that although techniques for gene therapy hold "great promise" for treatment, "there is still no conclusive evidence that a gene therapy protocol has been successful in the treatment of a human disease." Applicants respectfully submit that this invention differs markedly from gene therapy.

In this invention, sequence non-specific double-stranded polynucleotides are shown to activate the expression of immune recognition molecules in cells. It provides for a simple and specific system to activate expression of Class I and/or Class II molecules of the major histocompatibility complex (MHC), and allows regulation of expression of MHC molecules on the cell-surface of antigen presenting cells and other immune cells. The instantly claimed invention does not provide for "gene transfer and expression in human patients," as gene therapy is described in the French Anderson article cited by the Examiner. In fact, the sequence of the polynucleotides used in this invention is irrelevant to the claims of the invention.

B. Nonstatutory Subject Matter Rejection

The Examiner rejected Claim 46 under 35 U.S.C. § 101 as being directed to nonstatutory subject matter. The Examiner stated that the "terminology used in Claim 46, 'mammalian cell derived from a host organism', encompasses cells as implanted in a human or a human who has been made transgenic by the presence of such constructs, as well as the human thereof." The

Examiner further stated that "[c]laims directed to or including within its scope a human, will not be considered patentable subject matter under 35 U.S.C. § 101."

Applicants have amended claim 46 and respectfully submit that Claim 46 as amended is presently in condition for allowance.

C. Indefiniteness Rejection

The Examiner rejected Claims 40, 46 and 74 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regards as the invention.

Claim 40

Claim 40 was withdrawn from consideration in an election response to a restriction requirement.

Claim 46

The Examiner rejected Claim 46 "for use of the language 'capable of,' which is considered indefinite, since the language 'capable of' is considered to be drawn to some conditional function which is a latent characteristic, the scope of which is unclear in the claims." The "capable of" language has been eliminated from the claim.

The Examiner further stated that Claim 46 was rejected because it is "unclear how the antigen presenting cell would have the ability of increasing antigen presentation by a mammalian cell derived from a host organism." While applicants disagree with the Examiner, in order to eliminate any confusion, applicants have amended claims 46 and 74 and added new claim 76.

Support for these amendments and new claim can be found in the specification in Example 1 beginning on page 46, which teaches that any sequence non-specific double stranded polynucleotide increases MHC gene expression. Further support for this claim is found in

Example 2 beginning on page 60, which teaches that the cellular response to introduction of a double stranded polynucleotide is not limited to the MHC genes, but also reaches other genes important in antigen presentation. Additional support comes from Example 5 wherein somatic cells overexpressing the TSHR are treated with the sequence nonspecific double stranded polynucleotide, mitomycin killed, used to immunize animals, and produce an autoimmune disease, in this case Graves' . Although a disease is created in this model, the fundamental point that the cells so treated can present antigen in vivo to host immune cells is established. It is self-evident in this experiment, that an inherent property of the example provided is that the double stand polynucleotide-treated cells are capable of eliciting an in vivo immune response; the immune response can be custom tailored to the overexpressed antigen which may, for example be derived from a tumor cell or its RNA. Applicants respectfully submit that Claim 46 as amended is presently in condition for allowance.

Claim 74

The Examiner rejected Claim 74 asserting that it was unclear whether the method of increasing presentation of antigen by a cell derived from a host organism "mean[t] that the double-stranded polynucleotide would be introduced into the cell *in vitro* or *in vivo*."

Claim 74 has been amended by reciting the language that the double stranded polynucleotide is introduced to the mammalian cell "*ex vivo*." This change removes the uncertainty of how the method of increasing antigen presentation is conducted. Claim 74 was further amended by deleting the language "derived from a host organism" as a modifier to "a cell" because the inclusion of "ex vivo" reaches the same result with more clarity. A similar modification was made in Claim 46, and similar language was followed in new Claim 76, in order to maintain consistency and to present those claims in condition for allowance.

Support for this amendment can be found in the specification on page 30, line 23 through page 31, line 1, which states:

> "Increasing the ability of a cell to present antigen and activate the immune system by this invention allows its use as an activated APC. The activated APC may be introduced into an organism, preferably the activated APC is injected or surgically implanted into its own host organism (e.g., a murine cell into a mouse), to initiate an immune response."

Applicants respectfully submit that the rejection of Claim 74 for indefiniteness has been overcome and is presently in condition for allowance.

D. Enablement Rejection

The Examiner rejected Claims 1-35, 42-46, 60-66 and 74-75 under 35 U.S.C. § 112, first paragraph, stating that the specification:

> "does not reasonably provide enablement for a method of increasing the expression of any immune response molecule of any origin, in any mammalian cell by introducing any double-stranded polynucleotide into the cell comprising, activating expression of any gene or gene products involved in antigen presentation, growth, and function of the cell, and increasing the ability of the cell to present antigen to any immune cell."

Claim 1

Claim 1 has been amended in several respects. First, the limitation of Claim 3 has been imported into Claim 1. This change limits "double stranded polynucleotide[s]" to those which are "greater than 25 nucleotides in length," and clarifies that the sequence of the polynucleotide is non-specific. Similar modifications were made in Claims 46, 60, 74 (the other independent claims in the application), and similar language was followed in new Claim 76, in order to maintain consistency and to present those claims for allowance.

Support for this amendment can be found on page 141, lines 3-5 of the disclosure which states: "The polynucleotide can have a minimal length and activates the expression of molecules not encoded by a *nucleotide sequence that is not necessarily related to the polynucleotide*" (emphasis added). Additional support for the amendment can also be found on page 30, line 8-9

of the disclosure which states: "Thus the sequence of the polynucleotide is not necessarily related to any of the immune recognition molecules being activated."

Claim 1 has been further amended by adding the phrase "gene, or gene and product, or gene product" which is activated by the polynucleotide is "consisting of TAP-1, TAP-2, a proteosome subunit, Class II regulatory genes and gene products consisting of HLA-DM and invariant chain, costimulatory molecules gene or gene products consisting of B7 costimulatory molecule, PKR, IFN-beta, MAP Kinase, NF-κB, JAK, and a STATs." This limitation was imported from Claim 19, now cancelled. Similar modifications were made in Claims 46 and 74 in order to maintain consistency and to present the claims in condition for allowance.

Support for this amendment can be found in the specification in Experiment 2, which was performed to evaluate the effect of double stranded polynucleotides on the expression or activation of genes important for antigen presentation as well as MHC expression. Each member of the group of genes or gene products which are activated by double stranded polynucleotides can be found page 60, line 20 to page 61, line3; page 63, line 12 to page 64, line 16; and/or page 67, line 20 to page 69, line 20. Further support for these amendments can also be found in Example 3, beginning on page 71.

The Examiner also stated that although the claims are directed to any mammalian cell, the specification only teaches activation of immune response recognition molecules in the FRTL-5 mammalian thyroid cell. Applicants respectfully disagree and submit that the disclosure is much more complete. The disclosure teaches the use of cells of many different origins in several species. It teaches that double stranded polynucleotides induce an increase in the expression of immune response recognition molecules in "muscle cells, endothelial cells, fibroblasts, and

endocrine cells, i.e., thyrocytes, pancreatic islet cells and anterior pituitary cells ... lymphocytes, macrophages, dendritic cells" (See page 37 of the application).

Specifically, the specification states on pages 56 to 57 (a similarly detailed disclosure is also made on page 48, line 17 to page 50, line 24 of the application):

> "These results were not limited to rat FRTL-5 thyroid cells but were duplicated in a *human hepatoblastoma cell line, HuH7, in primary cultures of rat and human pancreatic islet cells, in primary and continuous cultures of human and mouse fibroblasts, in NIH 3T3 cells, in SkMC human muscle cells, in HUVEC human endothelial cells, in C2C12 mouse smooth muscle cells, in C34 mouse myoblast cells, in C57B16 spleen-derived dendritic cells in the WEHI231 Pre B cell line, in the P381D1 macrophage line, and in primary cultures of mouse spleen dendritic cells, mouse peritoneal macrophages, and mouse spleen macrophages.* In each case there was an increase in class I and class II RNA levels and in MHC antigen presentation measured by FACS analyses, albeit this was less dramatic in the immune cells where constitutively high levels of MHC class I, MHC class II, or both exist, e.g. C57 B16 dendritic cells, the P381D1 macrophage line, and in primary cultures of mouse spleen dendritic cells, mouse peritoneal macrophages, and mouse spleen macrophages. *In sum, the phenomenon was not cell specific.*" (Emphasis added).

Thus, the application teaches the use of many cell types in several places. It also gives specific guidance providing a working example of many other cell types.

The Examiner further stated that the specification does not enable such methods "by *ex vivo* gene therapy." To support the statement, the Examiner included an article published in Nature in April 1998 by French Anderson and commented that "there is still no conclusive evidence that a gene therapy protocol has been successful in the treatment of a human disease." Based on the foregoing, the Examiner concluded that the method of the instant application would have been "unpredictable" at the time of filing and would have required "undue experimentation." The Examiner stated that "there was no confirmed success in any human gene therapy trial, including trials involving the method of preventing or alleviating disease disorders in a mammalian subject, in vivo or ex vivo, by administering a double-stranded polynucleotide to

extracted mammalian cells, before immunizing the mammalian subject with an effective amount of the transfected, killed cells."

As noted above, however, applicants respectfully submit that this invention does not describe or claim methods for gene therapy in humans or otherwise. The Anderson article describes gene therapy as "gene transfer and expression" in a patient. The embodiment of this invention does not anticipate such an activity. As such, applicants respectfully submit that the rejection for lack of enablement based on a failure to enable gene therapy is overcome and applicants respectfully submit that Claims 1-35, 42-46, 60-66 and 74- are presently in condition for allowance.

In conclusion, Claim 1 has been sufficiently narrowed to more specifically tailor the claim to the disclosure as to both the type of double stranded polynucleotide and to the type of gene or gene product activated in response. Further, it has been shown that the specification teaches the use of sufficient cell types to justify granting the claim as written. Applicants respectfully submit that the rejection of Claim 1 for lack of enablement has been overcome.

Claims 2-28 and 42-45

Claims 2-28 and 42-45 were also rejected for lack of enablement. Claims 2-26 and 42-45 depend either directly from Claim 1, or from a claim which itself depends from Claim 1. Applicants submit that because the rejection of Claim 1 for lack of enablement has been overcome, the rejection for these dependent claims (excluding Claims 3, 19 and 20 which have been cancelled) has likewise been overcome.

Claims 27 and 28 have previously been withdrawn. No action is requested on these claims.

Claims 29-35 and 74 and 75

Claims 29-35, 74 and 75 were also rejected for lack of enablement. Claim 74 is an independent claim. Claim 74 has been amended as described above in order to overcome the 35 U.S.C. § 112, second paragraph rejection for indefiniteness. Claim 74 and its dependent claims are directed to methods for increasing presentation of antigen. Claim 74 was also amended to bring it into compliance with the amendments made to Claim 1 to overcome the enablement rejection. In light of the amendments, and pursuant to the reasoning above for Claim 1, applicants believe that the enablement rejection is overcome for Claim 74 and its dependent claims, Claims 29-35 and 75 and as such Claims 29-35, 74 and 75 are presently in condition for allowance.

Claim 46

Claim 46 was also rejected for lack of enablement. Claim 46 has been amended as described above in order to overcome the 35 U.S.C. § 101 rejection for being directed to non-statutory subject matter, and the 35 U.S.C. § 112, second paragraph rejection for indefiniteness. Claim 46 is directed to a somatic mammalian cell with the enhanced ability to present antigen. Claim 46 was also amended to bring it into compliance with the amendments made to Claim 1 to overcome the enablement rejection. In light of the amendments, and pursuant to the reasoning above for Claim 1, applicants believe that the enablement rejection of Claim 46 has been overcome and is presently in condition for allowance.

Claims 60-66

The Examiner additionally stated that the "specification [does not] provide enablement for a method of treating a mammalian disease sensitive to immunotherapy, such as cancer, nor

wherein the mammalian disease is an intracellular infection caused by virus, bacteria, yeast or protozoa, nor wherein the mammalian disease is caused by environmental injury."

The Examiner further stated that:

> "[t]he specification fails to provide an enabling disclosure for the method of treating a mammalian disease sensitive to immunotherapy, such as cancer, by removing diseased cells from a mammal, introducing a double-stranded polynucleotide into the cells, killing the cells, and immunizing the mammal with an effective amount of cells to prevent or alleviate the symptoms of the disease."

Specifically, the Examiner added that there was no "enabling disclosure for a double stranded nucleotide to be introduced into the diseased cells." Furthermore, the Examiner added that the specification failed to teach:

> "conclusively which double-stranded polynucleotide would be utilized to treat which specific mammalian disease. Likewise, no teachings are present in terms of how the extracted cells or transfectants would be killed, and how much of the transfectants, at what dosage, in what pharmaceutically acceptable carrier, which when administered to a mammalian subject, would be therapeutically effective in preventing or alleviating symptoms of the disease."

Claim 60

Claim 60 is directed to a method for treating a mammalian disease which is sensitive to immunotherapy. Claim 60 has been amended to recite that the mammalian disease claimed for treatment is "cancer, or an infectious disease caused by cancer, or a virus, bacteria, yeast, protozoa, a disease caused by environmental injury or an autoimmune disease." This recitation has been imported into Claim 60 from Claims 61 (cancer), 63 (intracellular infectious disease) and 65 (environmental injury). Claims 61 and 63-66 have been cancelled to reflect this change. Similar language was followed in new Claims 76 and 77 in order to maintain consistency and to present those claims in condition for allowance.

Support for Claim 60 as amended can be found in the specification on page 30, line 23 through page 31, line 1, which states:

"Increasing the ability of a cell to present antigen and activate the immune system by this invention allows its use as an activated APC. The activated APC may be introduced into an organism, preferably the activated APC is injected or surgically implanted into its own host organism (e.g., a murine cell into a mouse), to initiate an immune response."

Additional support for Claim 60 can be found on page 117, line 20-25 of the disclosure which teaches the specific example of a viral infection as follows:

"it was not surprising that transfection of the cytomegalic virus (CMV) promoter, pRcCMV, into FRTL-5 thyroid cells significantly increased class I RNA levels (Fig. 18, Row 2). More interestingly, however, we noted a coincident increase in 90K RNA levels (Fig. 18, Row 1), particularly in TSH treated (6H) cells. Similar results were obtained with plasmids containing SV40 and HIV promoters."

Furthermore, page 120, line 6-11 discloses that the same is true in the case of cancer:

"The ds nucleic acids would induce a controlled immune response, similar to a viral infection, causing bystander activation of the immune system. This could induce tumor cell destruction by cytotoxic immune cells or antibody mediated destruction. The ds nucleic acids become a means of therapeutic immuno-intervention to enhance tumor rejection by bystander activation of dormant autoreactive cells."

In addition, the specification provides the specific example of a Graves' disease model. In Example 5, applicants show that:

"Immunizing mice with the dsDNA-transfected hTSHR DAP.3 cells results, therefore, in the same Graves' like picture as previously described using cells expressing TSHR plus aberrant Class II. The dsDNA, by increasing Class I and Class II expression, duplicates the effect of aberrant Class II created by genetically overexpressing the Class II gene."

***

"We now show that ds DNA can increase MHC class I and class II antigen expression and increase expression of genes encoding proteins important for antigen presentation in fibroblasts. We show that immunization of dsDNA-transfected fibroblasts which also contain the hTSHR results in the development of exactly the same Graves' disease-like syndrome as hTSHR transfected RT4.15HP fibroblasts genetically engineered to aberrantly express MHC class II genes. Thus, we establish that transfection of dsDNA not only mimics the action of viral infection and viral DNA (Example 1), it can be the intermediate event in developing an autoimmune disease."

***

"In short, in these experiments, there is no question that dsDNA transfection provides the full array of antigen presenting molecules needed for the autoimmune response, as well as increased MHC class I and aberrant class II."

***

"[A]ny ds nucleic acid fragment, introduced in the cytoplasm by infection or leakage of self DNA, can directly induce MHC expression, and, concomitantly, increase or activate other essential factors important for antigen presentation. This can turn normal cells into antigen presenting cells with abnormally expressed MHC genes and thereby enable them to present auto- or foreign-antigens to our immune cell repertoire. This may be induced by viral DNA, ds viral RNA produced during the replication of RNA viruses, or perhaps viral- or environmentally-induced tissue damage."

***

"In summary, the present data offer the novel result that ds nucleic acids, by increasing MHC gene expression and the expression of antigen presenting genes can cause a cell with a functional TSHR to induce an autoimmune response, mediated by the normal T and B cell population. The disease mimics the major features of anti-TSHR receptor autoimmunity expressed in Graves' disease and supports the thesis that a primary viral or environmental insult of the target tissue, using this pathway, can induce autoimmune disease."

Importantly, the many particular details on these experiments are specifically taught in the Example, pages 89-109.

In conclusion, Claim 60 has been sufficiently narrowed to more specifically tailor the claim to the disclosure as to the type of diseases which are amenable to treatment using the methods claimed. Thus, applicants respectfully submit that the rejection of Claim 60 for lack of enablement has been overcome and is presently in condition for allowance.

Claims 61-66

Claims 61 through 66 were also rejected for lack of enablement. Applicants have cancelled Claims 61, 63-66. Claim 62, the sole remaining claim, depends from Claim 60's method of treatment, and is directed to use of the method as an adjuvant to enhance other treatment methods.

The Examiner stated that the "specification fails to provide an enabling disclosure for the method of treatment involving an adjuvant." The Examiner also stated that the application "fails to identify what would be an appropriate adjuvant, or whether or not conventional chemotherapeutic agents or treatment regimens, which when combined with the present invention, would be therapeutically effective in preventing or alleviating symptoms associates with a particular cancer, or other recited mammalian diseases."

Claim 62 has been amended, changing the language of the claim to read "the method of treatment is used to enhance other treatment methods," as opposed to "the method of treatment is used as an adjuvant to other treatment methods." Applicants respectfully submit that this change eliminates the uncertainty in any claim created by use of the word "adjuvant," helping to clarify that the claim is directed to the methods wherein the method recited in Claim 60 is used in addition to other methods of treatment. Similar language was followed in new Claim 78 in order to maintain consistency and to eliminate the possibility of a rejection of those claims on the same basis.

Support for this amendment is found on page 32 of the specification, which states:

> "The present invention may be used additively or synergistically with synthetic ODN expressing stimulatory CpG motifs, for example as adjuvants to boost the immune response to DNA and protein based immunogens and when coadministered with protein or DNA-based vaccines."

In light of the amended language of Claims 60 and 62, applicants respectfully submit that the rejection of Claim 62 for lack of enablement has been overcome and that Claims 60 and 62 are presently in condition for allowance.

E. New Claims

Claims 76-80 have been newly added. Claim 76 is directed to a vaccine for the treatment of disease. Support can be found in the specification on page 30, line 23 through page 31, line 1, which states:

> "Increasing the ability of a cell to present antigen and activate the immune system by this invention allows its use as an activated APC. The activated APC may be introduced into an organism, preferably the activated APC is injected or surgically implanted into its own host organism (e.g., a murine cell into a mouse), to initiate an immune response."

Claim 77 is directed to a method for treating specific diseases. Claims 78-80 depend from Claim 77. Support for Claims 77-80 can be found in the specification at the same locations as the support for treatment method Claim 60, as described above. Applicants respectfully submit that Claims 76-78 are presently in condition for allowance.

F. Closing

The Claims have been amended to clarify the claim language and to more particularly point out applicants invention. No new matter has been added. In view of the foregoing remarks and amendments, it is submitted that none of the rejections can be sustained and that all should be withdrawn.. Claims 1-35, 42-46, 60-66, 74, and 75, and new Claims 76-80 are in condition for allowance; reconsideration and allowance are respectfully requested.

Applicants respectfully remind the Examiner that the Claims 36-41, 47-59, 67-73 have been withdrawn from consideration by election in response to the Restriction Requirement mailed December 30, 1999.

If any matter requires attention prior to the allowance of the application, the Examiner is requested to contact the undersigned to resolve such matters.

Respectfully submitted,

Gerard P. Norton

Gerard P. Norton
Registration No. 36,621
Attorney for Applicant
Clifford Chance Rogers & Wells
200 Park Avenue
New York, New York 10166
(212) 878-8000

I hereby certify that this paper is being deposited this date with the U.S. Postal Service as first class mail addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Gerard P. Norton 10/25/00

Gerard P. Norton Date
Reg. No. 36,621

MAILING ADDRESS
Gerard P. Norton
CLIFFORD CHANCE ROGERS & WELLS L.L.P.
200 Park Avenue
New York, NY 10166
Telephone: (212) 878-3148